ACUTE ORAL TOXICITY STUDY IN RATS

[redacted]

FINAL REPORT

CENTER OF TOXICOLOGY AND PRECLINICAL SCIENCES
QUEST PHARMACEUTICAL SERVICES TAIWAN CO., LTD.

Signature Page

Study Director

Yu-Hui Yang, MS	Date

Facility Management

Hsiao-Lin Chen, ScD	Date

STUDY COMPLIANCE STATEMENT FOR GOOD LABORATORY PRACTICE

This study was conducted in accordance with protocol (Appendix I). All procedures have been conducted according to the Standard Operating Procedures. This report represents a true and accurate record of the results obtained.

This study was conducted in compliance with the Good Laboratory Practice (GLP) regulations set forth by (1) U.S. Food and Drug Administration (1987), Good Laboratory Practice Regulations, 21 CFR Part 58, (2) Department of Health, R.O.C. (2006), Good Laboratory Practice for Nonclinical Laboratory Studies, (3) Organization for Economic Co-operation and Development (OECD) (1997) Principles of Good Laboratory Practice (ENV/MC/CHEM (98)17) and (4) ISO/IEC (2005) General Requirements for the Competence of Testing and Calibration Laboratories (ISO/IEC 17025), with the exception of test article characterization and related analyses (supplied by the sponsor).

Yu-Hui Yang, MS
Study Director

Date

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APPENDICES

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[redacted]

1. ABSTRACT

The purpose of this study was to evaluate the 14-day acute toxicity of [redacted] in rats via single oral administration, to classify the test article according to the Globally Harmonised System (GHS) and determined the harmonized LD_{50} cut-off values. The study was designed in accordance with the OECD guideline for testing of chemicals #423.

The test article was homogenized in 0.5% (w/v) Methyl Cellulose to the concentration of 100 mg/mL. The dosing formulation was freshly prepared on the dosing day. Three female animals were used for the starting dose of 2000 mg/kg. Because neither mortality nor body weight loss over 20% was observed within 3 days, three more females were administered at the same dose. Animals were fasted overnight and were administered once with dose volume of 20 mL/kg. The animals were observed at approximately 0.5, 1, 2, 3 and 4 hours after dosing, followed by observing once daily for clinical signs and twice daily for mortality, and continued till Day 14. The body weight was recorded on the dosing day (D1), D4, D8 and at the end of the study period (D15). The animals were euthanized by exposure to carbon dioxide before exsanguination and necropsy.

After the animals were administered to the dose level of 2000 mg/kg, neither animal death nor clinical sign was found during the entire study period. The body weights were increased during the study period. At animal necropsy, no gross lesion was found in all rats.

Based on the data obtained from this study, the test article of [redacted] would be assigned to GHS Category 5/unclassified and the harmonized LD_{50} cut-off values is 5000 mg/kg.

2. PURPOSE

The purpose of this study is to evaluate the 14-day acute toxicity of [redacted] in rats via single oral administration. The results generated from this study allowed the test article to be ranked and classified according to the Globally Harmonised System (GHS) and determined the harmonized LD_{50} cut-off values.

The study was performed in accordance with the OECD guideline for testing of chemicals #423: Acute Oral Toxicity – Acute Toxi Class Method.

3. GENERAL INFORMATION

3.1 Sponsor

3.1.1 Name: [redacted]

3.1.2 Address: [redacted]

3.1.3 Representative: Annie [redacted]
Phone: + [redacted]
Fax: [redacted]
Email: [redacted]

3.2 Testing Facility

3.2.1 Name: Center of Toxicology and Preclinical Sciences, Quest Pharmaceutical Services Taiwan Co., Ltd. (CTPS, QPS Taiwan)

3.2.2 Address: 103, Lane 169, Kangning St., Xizhi Dist., New Taipei City 22180, Taiwan, R.O.C.

3.2.3 Location of study: Laboratory of testing group and the animal rooms C438 and C411

3.3 Personnel

3.3.1	Study director	Yu-Hui Yang, MS Phone: +886-2-2695-7767 Ext. 611 Fax: +886-2-6615-9989 Email: Yuhui.Yang@qps-taiwan.com.tw
3.3.2	Study veterinarian	Chih-Min Tseng, DVM, MS

3.4 Study Schedule

3.4.1 Experimental starting date (animal selection date): Feb. 21, 2011

3.4.2 Animal dosing dates: Feb. 22 and 25, 2011

3.4.3 Animal necropsy dates: Mar. 08 and 11, 2011

3.5 Data Retention

All raw data, documentation, records, protocol and a copy of the Final Report generated as a result of this study will be inventoried and retained in the archives of CTPS, QPS Taiwan for at least two years following the issuance of the Final Report.

The reserved test article was retained in the archives of CTPS, QPS Taiwan until the expiration date.

Statement: The test results relate only to the items tested; the final report shall not be reproduced except in full, without the written approval of the laboratory.

4. MATERIALS AND METHODS

4.1 Test Article (Appendix II)

4.1.1	Name	[redacted]
4.1.2	Date of receipt	Jan. 27, 2011
4.1.3	Source	Supplied by sponsor
4.1.4	Batch/Lot number	SCT501-1101
4.1.5	Code	TA01052
4.1.6	Ingredient	[redacted]
4.1.7	Physical appearance	off-white powder
4.1.8	Solubility	Insoluble in H_2O
4.1.9	Storage conditions	Room temperature
4.1.10	Expiration date	Jan. 24, 2013
4.1.11	Handling precautions	Gloves, mask, protective eyewear and lab coat were worn during formulation preparation and dosing.

4.2 Vehicle

The Methyl Cellulose (MC, Sigma, Lot no.: 076K0120) at 0.5% (w/v) was used for dosing formulation preparation.

4.3 Dosing Formulation Preparation

The test article was homogenized with MC and suspended in water for injection (WFI, Taiwan biotech Co., Ltd., Lot no.: 4BK1856) to the concentration of 100 mg/mL in 0.5% (w/v) MC. The test article formulation was freshly prepared on the dosing day and kept homogeneous prior to dosing.

4.4 Test System

4.4.1 Selection of animal

4.4.1.1 Species: Rats

4.4.1.2 Strain: Crl:CD (SD)

4.4.1.3 Source: BioLASCO Taiwan Co., Ltd., Taipei, Taiwan

4.4.1.4 Age and body weight range: nine weeks old and 227 to 235 grams at animal selection.

4.4.1.5 Justification for selection: The rat is the preferred rodent species according to the OECD guideline and with documented susceptibility to a wide range of toxic substances.

4.4.2 Preparation of animals

4.4.2.1 Quarantine and acclimation

Animals were quarantined (in room C431, see Appendix III) and acclimated for 6 days prior to study.

4.4.2.2 Method of identification: Ear-notch and cage tags were used for animal identification. Each cage was identified by a card indicating cage number, study number, dose level, sex and animal IDs.

4.4.2.3 Number and sex: A total of 6 females were used in this study.

4.4.2.4 The IACUC animal study protocol approval number is CTPS-11-004.

4.4.3 Animal care

4.4.3.1 Housing conditions

The animals were housed individually in a Polycarbonate (PC) cage (three rats per cage) in the AAALAC accredited animal facility. The animals were housed in room C438 for three days, then transferred to room C411 due to the air pressure problem. The daily mean temperature was 20.6 ~ 21.3 ℃ in C438 and 20.5 ~ 21.5 ℃ in C411 (Appendix IV). The daily mean relative humidity was 50.8 ~ 57.3% in C438 and 29.2 ~ 58.6% in C411 (Appendix V). The 12-hr/12-hr light/dark cycle with light on at 6:00 AM and off at 6:00 PM was maintained.

4.4.3.2 Diet

Laboratory Autoclavable Rodent Diet 5010 (PMI® Nutrition International, Inc., MO, USA) was supplied *ad libitum* throughout the entire study period (Appendix VI). The nutrients analysis was supplied by the manufacturer (Appendix VII). The diet is routinely analyzed for microbes and agriculture chemical contaminants. The contaminants analysis records are maintained in the archives of CTPS, QPS Taiwan.

4.4.3.3 Water

Polycarbonate water bottles were filled with water supplied by the Taipei Water Department, autoclaved, and then attached to the cages. The water was supplied *ad libitum* throughout the entire study. The water is routinely analyzed for specific microbes and contaminants. The contaminants analysis records are maintained in the archives of CTPS, QPS Taiwan.

4.5 Experimental Design

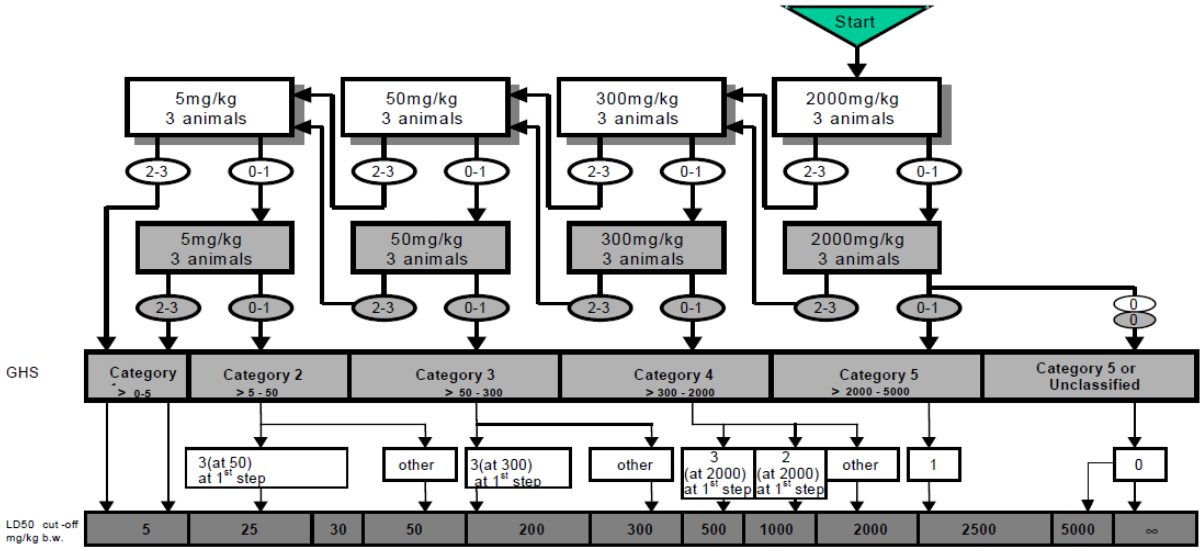
4.5.1 Animal selection

Before dosing, all animals were weighed and observed for clinical signs. Six animals were selected in the study. The weight variation between two groups of animals did not exceed ± 20% of the mean body weight of each other.

4.5.2 Study design

Three female animals were used for the starting dose of 2000 mg/kg. Because neither mortality nor body weight loss over 20% was observed within 3 days, three more females were administered at the same dose. Total 6 animals were administered test article formulation at dose of 2000 mg/kg with concentration of 100 mg/mL. The dosing volume was 20 mL/kg. Based on flow charts of the

procedure as below, the classification in GHS and harmonized LD_{50} cut-off values of test article were determined.



4.5.2.1 Rationales for dose level selection

The information of similar compound supplied by sponsor suggests that mortality is likely at the dose level of 2000 mg/kg. Therefore, the starting dose of 2000 mg/kg was used.

4.5.3 Test article administration

4.5.3.1 Route: Oral gavage

4.5.3.2 Rationales for selection of the dosing route: The oral route is the incautious route for human take of the test article.

4.5.3.3 Frequency of administration: Once

4.5.3.4 Fasting: The animals were fasted overnight.

4.5.3.5 Dose volume: 20 mL/kg, the actual dosing volume was adjusted based on the individual body weight before dosing.

4.5.3.6 After the test article had been administered, food was withheld for a further 3-4 hours.

4.5.3.7 The dosing day was denoted as D1 (Day 1). No animal was replaced on the dosing day.

4.6 Observation and Examination

4.6.1 Observations of animals

The rats were observed for mortality and clinical signs at approximately 0.5, 1, 2, 3 and 4 hours after dosing. During the following days (D2 ~ D14), the animals were

observed twice daily (at least six hours apart) for mortality and once for clinical signs. Any clinical signs were recorded and documented.

4.6.2 Body weights

Body weight was recorded on animals treated with test article, prior to the start of dosing (D1), D4, D8, and at the end of the study period (D15). The total body weight changes (D15-D1) were calculated.

4.6.3 Gross necropsy

Gross necropsy was performed on all animals at the end of study (D15). The animals were euthanized by carbon dioxide exposure followed by exsanguinations and necropsied in a randomized order. The external surface of the body and all organs/tissues in the thoracic and abdominal cavities were examined and recorded.

5. RESULTS AND DISCUSSION

5.1 Mortality and Clinical Observations

The individual data of mortality and clinical observations are presented in Table 1.

Neither death nor clinical sign was found in all animals during the entire study period.

5.2 Body Weights

Individual data of the body weights and body weight changes are presented in Table 2.

The body weights were increased during the study period.

5.3 Gross Necropsy

A summary of the gross finding is presented in Table 3.

No gross lesion was found in all animals.

6. CONCLUSION

After the animals were administered to the dose level of 2000 mg/kg, neither animal death nor clinical sign was found during the entire study period. The body weights were increased during the study period. At animal necropsy, no gross lesion was found in all rats. Based on the data obtained from this study, the test article of [redacted] would be assigned to GHS Category 5/unclassified and the harmonized LD_{50} cut-off values is 5000 mg/kg.

7. REFERENCES

None

Table 1. Mortality and Clinical Observations

Animal ID.	Results
1110040003	No animal death and no clinical signs were observed
1110040007	
1110040009	
1110040001	
1110040002	
1110040005	

Table 2. Body Weights

Animal ID.	Body Weight (g)				Total Body Weight Changes (g)
	D1	D4	D8	D15	D15-D1
1110040003	221	245	252	259	38
1110040007	221	251	263	275	54
1110040009	216	241	250	259	43
1110040001	215	239	242	243	28
1110040002	224	249	254	263	39
1110040005	222	245	252	259	37

D15-D1: Body weight on D15 minus body weight on D1

Table 3. Gross Necropsy

Animal ID.	Results
1110040003	No gross lesion was observed
1110040007	
1110040009	
1110040001	
1110040002	
1110040005	